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Effect of Stobadine on Leukocyte Free Radical Generation in Streptozotocin-Diabetic Rats: Comparison with Vitamin E

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Chemiluminescence · Streptozotocin · Stobadine · Vitamin E

Abstract
We investigated a possible alteration in the ability of leukocytes to produce reactive oxygen species by stobadine, a pyridoindole antioxidant, in streptozotocin-diabetic rats. The production of free radicals from whole blood was assessed by luminol-enhanced chemiluminescence after stimulation by phorbol myristate acetate. The effects of vitamin E treatment were also evaluated and compared with the effects of combined treatment with stobadine. Diabetes was induced by streptozotocin (55 mg/kg i.p.). Some of diabetic rats and their age-matched controls were treated orally with a low dose of stobadine (24.7 mg/kg/day), vitamin E (400–500 IU/kg/day), or stobadine plus vitamin E for 10 weeks. Stobadine and vitamin E separately produced, to a similar degree, a reduction in diabetes-induced hyperglycemia. The phorbol myristate acetate stimulated chemiluminescence signal was markedly depressed in both moderate and severe diabetic rats. Stobadine treatment prevented this depression of the chemiluminescence response. Vitamin E treatment also eliminated the depression of the chemiluminescence signal in diabetic rats, and the combination with stobadine did not produce further improvement in leukocyte function. These results suggest that stobadine treatment alone is able to produce beneficial effects on leukocyte function and to maintain leukocyte free radical release during diabetes.

Introduction

There is evidence that elevated levels of glucose result in impairment of the function of leukocytes in diabetes. Diabetes has been shown to result in a significant decrease in formyl-methionyl-leucyl-phenylalanine-induced chemiluminescence (CL) and in a significant increase in sorbitol in neutrophils of streptozotocin (STZ) diabetic rats [1]. Suppression of chemotaxis and phagocytosis has also been reported in leukocytes from diabetic patients [2]. It has been demonstrated, 4 days after STZ injection, that the blood glucose level was increased and that the luminol CL response was significantly reduced in diabetic rats, indicating that an increased susceptibility to bacterial infection in diabetic rats results from impaired neutrophil function to produce reactive oxygen species [3]. Thus, these data support results of reports showing that the host

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defense mechanisms become weakened and that the susceptibility to infections is increased in diabetic patients [2].

Stobadine, (–)-cis-2,8-dimethyl-2,3,4,4a,5,9b-hexahydro-1H-pyrido[4,3b]indole, was shown to scavenge reactive oxygen species such as hydroxyl, alkoxyl, and peroxyl radicals and to quench singlet oxygen to repair oxidized amino acids and to preserve oxidation of SH groups by one-electron donation. Against superoxide radical, however, it exhibited only a nonsignificant scavenging effect [4]. Although it has been demonstrated that stobadine dose-dependently decreased the CL generated from phorbol myristate acetate (PMA) or N-formyl-methionyl-leucyl-phenylalanine-stimulated (but not from A-23187-stimulated) human polymorphonuclear leukocytes [5, 6], the effect of stobadine on leukocyte free radical release in diabetes has not been investigated. Since different antioxidant compounds may act synergistically, and since some combinations may be more effective than any one compound alone, in the present study, the effects of stobadine plus vitamin E treatment were also evaluated and compared with the effects of treatment with each agent alone. Therefore, the aim of this study was to evaluate the effects of stobadine treatment on leukocyte free radical production in diabetic rats and to compare the response with vitamin E treatment by using a CL method.

Materials and Methods

Induction of Diabetes and Treatment Protocols

Male Wistar rats weighing 250–300 g were fed a standard rat chow diet and had access to water ad libitum. Diabetes was induced by a single intraperitoneal injection of STZ (55 mg/kg) to animals which fasted overnight. Diabetes was verified 48 h later by measuring tail vein blood glucose levels, and the rats with blood glucose concentrations of 300 mg/dl or more were considered diabetic. Two days after injection of STZ or vehicle, the rats were divided into the following groups: (1) untreated diabetic rats (n = 9); (2) diabetic rats treated with stobadine (24.7 mg/kg/day p.o.; n = 10); (3) diabetic rats treated with vitamin E (α-tocopheryl acetate, 400–500 IU/kg/day p.o.; n = 8); (4) diabetic rats treated with both stobadine and vitamin E as given in protocols 2 and 3 (n = 10); (5) untreated control rats (n = 5); (6) control rats treated with stobadine as given in protocol 2 (n = 5); (7) control rats treated with vitamin E as given in protocol 3 (n = 5), and (8) control rats treated with stobadine plus vitamin E as given in protocol 4 (n = 5). The dose regimen of stobadine or vitamin E was chosen according to a previous study [7]. The animals were treated for a period of 10 weeks, beginning 48 h after either vehicle or STZ injection. Blood glucose concentrations were measured by an Accutrend® GCT meter (Roche Diagnostics, Basel, Switzerland). The principles of laboratory animal care [NIH publication No. 85–23, revised 1985] were observed.

PMA-Induced CL

Freshly obtained rat whole blood (50 μl) was diluted with Hanks' balanced salt solution containing 1 mmol/l calcium (860 μl, pH 7.4) in a cuvette (total volume of 1 ml), and 40 μl luminol (100 μmol/l, final cuvette concentration) was added. Then stimulant PMA (50 μl) was added to yield a final cuvette concentration of 5 μmol/l. The luminol CL was measured at 37°C using a model 1250 chemiluminometer (BioOrbit, Turku, Finland). The CL produced was measured continuously and recorded on a computer by using the luminometer 1250 program (version 1.12; BioOrbit) for 15 min.

Drugs

All chemicals except stobadine were purchased from Sigma Chemical (St. Louis, Mo., USA). Stobadine dipalmitate was obtained from the Slovak Academy of Sciences (Bratislava, Slovak Republic). Luminol was prepared daily in 2 mol/l NaOH (2.5%) and diluted with phosphate-buffered saline (KH_2PO_4 10 mmol/l and NaCl 150 mmol/l, pH 7.4) before use.

Statistics

Data are expressed as mean values ± SEM. They were first subjected to Bartlett's test for homogeneity of variances and were given a log transformation if necessary. One-way analysis of variance was then performed, followed by the Student-Newman-Keuls test to estimate the significance of differences for individual between-group comparisons. For the statistical evaluation of starting and final blood glucose concentrations within groups, Student's t test was used. $p < 0.05$ was considered to denote statistical significance of differences.

Results

The final blood glucose concentrations of untreated diabetic rats were about four times higher than in normal control rats (table 1). Treatment with stobadine alone, vitamin E alone, or stobadine in combination with vitamin E did not markedly modify the blood glucose levels in control rats. Stobadine either alone or in combination with vitamin E produced significant reductions in blood glucose levels in diabetic rats. There was also a marked decrease in blood glucose levels in the vitamin E treatment group of diabetic rats. The magnitude of the blood glucose lowering effect of stobadine treatment was found to be comparable to the effects of vitamin E treatment. The combination of stobadine and vitamin E provided some further beneficial effect on blood glucose levels; nevertheless, at the end of the treatments, the diabetic rats were still hyperglycemic when compared with normal control rats (table 1).

Effects of Stobadine and Vitamin E on the Luminol CL in Rat Whole Blood

PMA (5 μmol/l) produced a signal of 0.48 ± 0.04 mV (n = 5) in the CL assay in the control group. This response is markedly depressed in blood obtained from diabetic

rats (0.24 ± 0.04 mV, n = 9, $p < 0.01$; fig. 1). Stobadine treatment prevented this impairment of the function of leukocytes in diabetic rats (0.47 ± 0.05 mV, n = 10, $p < 0.05$). Protective effects of stobadine were found to be similar to those of the vitamin E treatment in diabetic rats (0.53 ± 0.07 mV, n = 8, $p < 0.05$). The combination of stobadine and vitamin E treatment did not produce further improvement in leukocyte function in diabetic rats (0.48 ± 0.06 mV, n = 10, $p < 0.05$). In control rats, administration of stobadine and vitamine E alone, or in combination, produced similar responses to the signals obtained in the control group (fig. 1).

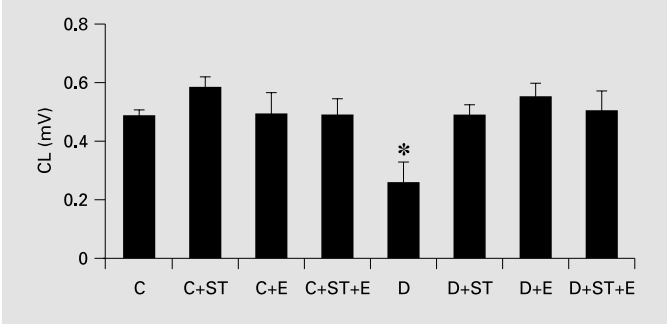

Fig. 1. Effects of stobadine (ST), vitamin E (E) or stobadine plus vitamin E (ST+E) on PMA (5 μmol/l)-induced luminol CL in whole blood obtained from STZ-diabetic (D) or control (C) rats. The numbers in controls (C, C+ST, C+E, and C+ST+E) were 5 in each group, and the numbers for D, D+ST, D+E, and D+ST+E groups were 9, 10, 8, and 10, respectively. The results were calculated as peak CL and are shown as mean values ± SEM. Duplicate assays were performed in all experiments. * $p < 0.05$ when compared with the other groups.

Table 1. Blood glucose levels (at the end of the experimental period) of control and diabetic animals untreated or treated with stobadine, vitamin E, or stobadine plus vitamin E

Groups	n	Blood glucose mg/dl
Untreated control rats (C)	5	108.2 ± 3.3
Control rats treated with stobadine (C + ST)	5	118.4 ± 5.9
Control rats treated with vitamin E (C + E)	5	104.0 ± 2.3
Control rats treated with stobadine plus vitamin E (C + ST + E)	5	100.0 ± 3.8
Untreated diabetic rats (D)	9	433.8 ± 18.8*
Diabetic rats treated with stobadine (D + ST)	10	348.8 ± 14.2*,+
Diabetic rats treated with vitamin E (D + E)	8	326.5 ± 12.4*,+
Diabetic rats treated with both stobadine and vitamin E (D + ST + E)	10	313.1 ± 12.4*,+

Data are reported as mean values ± SEM. The blood glucose concentrations of all diabetic animals were significantly different from those of the control animals untreated or treated with antioxidants.

* $p < 0.05$ when compared with the corresponding control groups; + $p < 0.05$ when compared with untreated diabetic rats.

Discussion

In the present study, we have obtained first experimental evidence that stobadine is able to inhibit suppression of leukocyte free radical generation in STZ-diabetic rats. This effect was comparable with that of the vitamin E treatment, but the combination of stobadine and vitamin E did not produce further improvement. The whole-blood assay used in our study provides a natural environment for neutrophils, avoids cell activation by isolation procedures, and prevents washing out of drugs used in vivo.

Our results indicate that oral administration of stobadine or vitamin E alone or in combination did not impair the polymorphonuclear neutrophil function in control rats. These observations are in agreement with the finding that the CL was not affected in premature infants and adults after oral vitamin E supplementation [8]. In another study [9], α-tocopherol supplementation for 3 months failed to exert any significant effect on either whole blood CL or migration of the patients' neutrophils. On the other hand, a decrease in the CL has also been reported in isolated leukocytes after oral supplementation with vitamin E in volunteers [10]. A transient inhibition of the PMA-activated luminol CL during administration of vitamin E was shown in another study [11].

Experimental diabetes in rats decreased the PMA-induced CL in the present study. Our results support previous observations showing that diabetes resulted in a significant decrease in the CL response [1, 3]. It has been reported that 4 days after STZ injection the blood glucose level increased and that the luminol CL response was significantly reduced in diabetic rats. We have observed a marked reduction in the CL at the end of the 10-week period of diabetes. An increase in blood glucose levels and a decrease in luminol CL have been determined 4 days after STZ injection in diabetic rats [3]. A significant increase in sorbitol in neutrophils from STZ-diabetic rats was observed, and this increase may involve the underlying mechanism of a reduction in leukocyte free radical release [1]. High levels of acetoacetate, a serum metabolite which is also elevated in ketosis, have also been suggested to be involved in the diminished phagocytic activity of human neutrophils [12].

Although it has been reported that stobadine has free radical scavenging properties both in vivo and in vitro [4] and that it inhibits STZ-induced lipid peroxidation [7], the mechanism of inhibition of the effects of diabetes on leukocyte free radical release is not known from the present study. We have shown that stobadine reduced hyperglycemia which may involve the restoration of the leukocyte CL response in our study. Stobadine has been shown to suppress the STZ-induced elevation of the blood glucose levels in rats [7] and the alloxan-induced hyperglycemia in mice in a dose- and time-dependent manner [13]. It has been demonstrated in a recent study [14] that stobadine was able to restore the enzymatic activities related to pentose phosphate pathway and glutathione-dependent metabolism in brain and peripheral organs of diabetic rats.

In conclusion, our results showed that stobadine was effective in the prevention of impairment of leukocyte free radical release function in STZ-diabetic rats. This protective effect is comparable with that of vitamin E treatment. Our results suggest that stobadine is capable of improving the neutrophil bactericidal function in diabetes which may reduce the incidence of clinical bacterial infections in diabetic patients.

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